enzymatic activity and appeared heterogeneous when subjected to paper chromatography.

In our attempt to rule out the possibility that the slower eluting peak might have resulted from concentration of impurities in the original enzyme solution, we performed the following experiment: A solution of crystallized pinguinain was prepared and subjected to gel-filtration in 'Sephadex *G*-100', in a column having dimensions of 40 × 4 cm, using 0·1 M acetate buffer *p*H 4·6 as eluting buffer. The two absorption peaks at 280 mμ appeared. The fractions containing the faster eluting peak were pooled, dialysed as already explained, and lyophilized. Solutions of the crystallized enzyme preparation and of the re-filtered and re-lyophilized material from the faster eluting peak were again subjected to gel-filtration. The two solutions yielded very similar elution patterns (Fig. 2). The slower eluting component reappeared in the crystallized material obtained from the faster eluting peak, thus verifying that the appearance of this component is due to a partial breakdown of some of the protein molecules caused by the crystallization and lyophilization process. Whenever crystallization under these experimental conditions occurs, the optical density ratio between the two components is about 2 : 1.

These results indicate that crystalline pinguinain can be obtained in very high yields using a rather simple method but that its lyophilization causes a partial breakdown of a certain proportion of the molecules. The loss of activity occurring after crystallization is reversible. Crystallization can be accomplished only in the presence of compounds containing free sulphydryl groups. Pinguinain seems to be composed of a single peptide chain, as shown by the production of a single DNP derivative.

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Occurrence of 5-Hydroxy-L-tryptophan as a Free Plant Amino-acid

Using labelled amino-acids and chromatographic techniques, it has been shown that 5-hydroxytryptophan is an intermediate in the conversion of tryptophan to the physiologically important base 5-hydroxytryptamine (serotonin) in higher animals[1,2]. The hydroxylation of tryptophan in the 5 position has also been demonstrated in *Chromobacterium violaceum*[3] and in tissue slices of *Citrullus vulgaris* (watermelon)[4].

In these animal, bacterial and plant cells, however, the concentration of 5-hydroxytryptophan is low and confirmation of its presence has rested on indirect evidence rather than on the isolation of the compound itself.

As part of a programme designed to investigate the distribution of 'non-protein' amino-acids in leguminous plants and investigate possible toxic or other physiological effects, which they may produce when eaten by man and higher animals, we have examined the seed of *Griffonia simplicifolia*, a plant of reputed medicinal value, the leaves of which are fed to sheep and goats in West Africa to stimulate reproduction[5]. Ethanolic (50 per cent) extracts of this seed were found to contain high concentrations (estimated at > 1 per cent dry seed weight) of a compound which reacted with ninhydrin, gave a blue-purple colour with Ehrlich's reagent and occupied an unusual position for a plant amino-acid on two-dimensional chromatograms. Tests with α-nitroso-β-naphthol[6] indicated that the compound was a 5-hydroxyindole, and the ultra-violet absorption maxima shown by partially purified aqueous solutions, at acid and alkaline values of *p*H, corresponded with those given in the literature for 5-hydroxytryptophan.

Extracts of the 'unknown' were chromatographed in seven different solvent systems and subjected to high-voltage ionophoresis on paper at four different values of *p*H using authentic samples of 5- and 6-hydroxy-D,L-tryptophan (kindly given by Dr. S. F. Contractor) as 'markers'. In all systems the 'unknown' moved with 5-hydroxytryptophan. In the solvent system previously used to resolve 5-hydroxy-D,L-tryptophan[7], and also in methanol : pyridine : water (20 : 5 : 1 by volume) which was found to separate the optical isomers equally well, the compound from *Griffonia simplicifolia* ran with the slower-moving 5-hydroxy-L-tryptophan. The colour given by the 'unknown' with Ehrlich's reagent was identical both in shade and in rate of development with that given by authentic 5-hydroxytryptophan, and a characteristic change of colour, from purple through green to brown, was observed when 'spots' of the plant amino-acid and of synthetic 5-hydroxytryptophan on paper chromatograms were developed successively with ninhydrin and Ehrlich's reagent.

After isolation and recrystallization the infra-red spectrum of the purified compound was found to be identical with that of synthetic 5-hydroxy-L-tryptophan (supplied by Calbiochem).

The quantitative recovery of this amino-acid from the seed, its distribution, biosynthesis, fate in the plant and the possibility of it being directly or indirectly responsible for the physiological effects attributed to *Griffonia simplicifolia* are at present being investigated.

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Acetate and Other Carboxylic Acids as Precursors of Ethylene

The fungus *Penicillium digitatum*, which is the common green mould of citrus fruit, produces a small amount of ethylene which is of physiological significance to its host. The mechanism of ethylene synthesis by the fungus and other plant tissues has been explored in several laboratories by supplying postulated precursors specifically labelled with carbon-14 or tritium. Burg[1] reviewed this work in 1962 and concluded that ethylene is "synthesized fairly directly from sugar, but the steps between glucose and the final product have completely evaded detection". Wang *et al.*[2] have fed specifically labelled sugars and acids to *Penicillium digitatum* and concluded that an "intimate